

Effect of Prostaglandin E in Multiple Experimental Models. VIII. Effect on Host Response to Metastatic Tumor

J. PAUL WAYMACK, MD, ScD, ELIEZER FLESCHER, PhD, JAYA VENKATRAMAN, PhD,
GABRIEL FERNANDES, PhD, REY F. GUZMAN, BS, ROGER W. YURT, MD,
ARTHUR D. MASON, JR., MD, AND BASIL A. PRUITT, JR., MD

From the U.S. Army Institute of Surgical Research, Ft. Sam Houston (J.P.W., R.F.G., A.D.M., B.A.P.), and Department of Medicine, University of Texas Health Science Center, San Antonio (E.F., J.V., G.F.); Department of Surgery, University of Cornell Medical Center, New York, New York (R.W.Y.)

Prostaglandin E (PGE) is produced by certain tumors and is reported to decrease primary tumor growth. We evaluated its effect in multiple tumor models utilizing a 1 week course of the long acting PGE derivative dimethyl-PGE (dPGE) at a dosage of 100 μ g/kg/day vs. a lactated Ringers control. For all tumor models, a suspension of 1×10^6 colon carcinoma cells were injected into Wistar-Furth rats. When the suspension was injected subcutaneously and the drug was begun at the time of tumor challenge, there was no effect on survival. When the tumor was injected intraperitoneally or intravenously and the drug begun at the time of tumor challenge, dPGE decreased survival time. When the tumor was administered intravenously but dPGE was delayed for 5 days, there was no effect on survival time. When rats were given a 1 week course of dPGE or saline, dPGE was found not to alter natural killer (NK) cell cytotoxicity, macrophage cytotoxicity, spontaneous lymphocyte blastogenesis, or mitogen stimulated blastogenesis. dPGE failed to alter lymphocyte metabolism of glucose in nonstimulated lymphocytes, but decreased the rate of glucose metabolism and adenosine deaminase activity in mitogen stimulated lymphocytes. In conclusion, PGE appears to enhance metastatic growth of tumor lines where it does not alter primary tumor growth. This effect does not appear immunologically mediated.

KEY WORDS: immunology, lymphocytes, NK cells, carcinoma

INTRODUCTION

Oncology patients have been demonstrated to have elevated levels of prostaglandin E (PGE) in their primary tumor tissue and systemic circulation [1]. The PGE has also been reported to exert autocrine, paracrine, and endocrine effects on both the tumor, and the host response to the tumor [2]. The majority of effects of PGE on tumor cells appear to be directed towards inducing differentiation of the tumor cell and to decreasing the rate of replication of the tumor [3,4]. As such, it has been hypothesized that elevated levels of PGE are beneficial to tumor bearing hosts.

PGE has also been hypothesized to exert detrimental effects on the host's immunologic response to the tumor.

This belief is based primarily on in vitro studies where leukocytes were cultured in the presence of PGE and were then assayed for various immune functions. These studies demonstrated that, in in vitro models, PGE impairs lymphocyte blastogenesis, natural killer (NK) cell cytotoxicity, macrophage cytotoxicity, and granulocyte chemotaxis [5-7]. There have, however, been very few studies evaluating the effect of PGE on immune

Accepted for publication August 6, 1991.

Address reprint requests to Library Branch, U.S. Army Institute of Surgical Research, FT. Sam Houston, San Antonio, TX 78234-5012.

The opinions and assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.

function in *in vivo* models or on tumor metastases. Herein we report results from such experiments.

MATERIALS AND METHODS

Animals

Adult male Wistar-Furth rats weighing approximately 250 g were used in all experiments. The rats were housed in individual cages and allowed food and water *ad libitum* throughout the study. All animals were observed for 1 week prior to entry into the study to exclude the possibility of any pre-existing diseases. The care of all animals was in accordance with the guidelines set forth by the Animal Welfare Act and other federal statutes and regulations relating to animals and studies involving animals, and with the guide for care and use of laboratory animals (National Institute of Health publication 86-23).

Drug Protocol

The long acting prostaglandin E derivative 16,16-dimethyl-prostaglandin E (dPGE) was administered intraperitoneally to half the animals in these studies at a dosage of 50 $\mu\text{g/kg}$ body weight, twice daily for a 7 day period. The remaining rats received lactated Ringers intraperitoneally on a twice daily basis for 7 days and served as controls.

Tumor Preparation

The tumor was obtained from a 1,2-dimethyl-hydrazine-induced, poorly differentiated syngeneic colon carcinoma. The tumor cell suspension utilized in these experiments was obtained by harvesting the tumor from a subcutaneous site after it had grown to approximately 50 g in size. The tumor was mechanically disaggregated by first slicing it into approximately 1 mm³ pieces and vigorously shaking the suspension in complete RPMI-1640 medium with penicillin, streptomycin, and 10% fetal calf serum. The cells were washed three times in the same media. A sample of the suspension was stained with trypan blue and the number of viable tumor cells determined. The cells were resuspended in sufficient RPMI media to achieve a final concentration of 1×10^6 cells/ml.

Tumor Protocols

Three tumor models were used to evaluate the effect of dPGE on host response. In the first model (primary tumor model), 40 rats (control = 20; dPGE = 20) had 1 ml of the tumor suspension (1×10^6 tumor cells) injected subcutaneously between the scapula. The animals received their initial dose of either lactated Ringers or dPGE immediately prior to being challenged with the tumor cell suspension. The animals were followed to death and mean survival times determined. This model

has previously been shown to result in a 100% mortality [8].

The second model (peritoneal carcinomatosis model) involved the injection of 1×10^6 tumor cells intraperitoneally immediately following administration of the initial dose of either dPGE ($n = 20$) or lactated Ringers ($n = 20$). The animals were again followed to death and mean survival times plus absolute survival rates determined.

For the third model (pulmonary metastases model), the animals received their initial dose of dPGE ($n = 20$) or lactated Ringers ($n = 20$) immediately prior to the tumor challenge. For this challenge, 1×10^6 of the tumor cells were injected intravenously through the dorsal penile vein. This model has previously been shown to result in a 100% mortality [8].

For the final protocol, 1×10^6 of the tumor cells were again injected intravenously (pulmonary metastases model). On this occasion the animals were not administered their initial dose of lactated Ringers ($n = 20$) or dPGE ($n = 20$) until 5 days following injection of the tumor cell suspension. These animals were followed to death and mean survival times determined.

Leukocyte Infiltration

Leukocyte infiltrates were determined for those animals in the pulmonary metastases challenge model where the initial dose of the drug was administered immediately prior to injection of the tumor. Necropsy was performed for these animals and biopsy specimens of the pulmonary metastases were obtained, fixed in formalin, and stained with hematoxylin and eosin for analysis of leukocyte infiltration. Leukocyte infiltrates were quantified by enumeration of white blood cells in the tumor periphery. Fifteen high power fields (hpf) were counted for each specimen with an image-analysis system (Optomax 40-10, Optomax Incorporated, Hollis, New Hampshire). An average was obtained for these 15 hpf.

Analysis of Immunologic Function

The effect of PGE on immunologic function was evaluated by administering a 1 week course of either 50 $\mu\text{g/kg}$ of dPGE or lactated Ringers twice daily for 7 days. At the end of this time, leukocytes were harvested and assayed for multiple immunologic parameters.

Harvesting Lymphocytes

At the completion of the 7 day course of dPGE ($n = 10$) or lactated Ringers ($n = 10$) the rats were sacrificed by decapitation. The spleens were aseptically removed and homogenized in RPMI-1640 supplemented with penicillin, streptomycin, and 10% fetal calf serum. The number of viable nucleated cells in each sample was

determined and appropriate dilutions performed to achieve the desired concentrations of lymphocytes.

Harvesting Macrophages

Elicited peritoneal macrophages were obtained by intraperitoneal administration of 4 ml of brain-heart infusate 4 days prior to sacrifice. Following 7 days of dPGE treatment ($n = 10$) or lactated Ringers ($n = 10$) the animals were sacrificed by decapitation. A midline celiotomy was performed aseptically and the peritoneal cavity lavaged with 20 ml of Hank's balanced salt solution (HBSS) without calcium or magnesium and with 0.25 mM EDTA. The resulting suspensions were hypotonically lysed of any contaminating red cells and then washed three times in standard HBSS. The number of macrophages present was determined with a hemocytometer, and the macrophages were resuspended in sufficient RPMI-1640 to achieve the desired concentration.

NK Cell Function

The NK cell function of the lymphocyte preparations was determined using a 100:1 effector/target cell ratio. The assay was performed in a volume of 0.2 ml per well of RPMI media containing 1×10^5 lymphocytes and 1×10^3 mouse Moloney virus-induced lymphoma cells (YAC cells) and target cells (100:1 effector/target cell ratio) which had previously been labelled with sodium ^{51}Cr chromate. After centrifugation at 40g for 2 minutes the plates were incubated at 37°C in 5% CO_2 for 4 hours. The plates were then centrifuged at 500g for 5 minutes and a 100 μl aliquot of the supernatant was collected and assayed for ^{51}Cr on a gamma counter. The percentage of cell lysis was calculated as mean counts per minute released in the presence of effector cells minus mean counts per minute spontaneously released by target cells incubated with media alone divided by the counts per minute released after treating cells with Triton X-100 (1:100 dilution) minus the counts per minute spontaneously released with media alone; the quotient multiplied by 100.

Macrophage Cytotoxicity

Macrophage cytotoxicity was assayed against YAC cells as follows. Briefly, 5×10^4 ^{51}Cr -labelled YAC cells were mixed with 5×10^5 macrophages in a total volume of 0.2 ml of RPMI media. The wells were cultured at 37°C in 5% CO_2 for 24 hours. The wells were then centrifuged and the supernatants from each well harvested and assayed for ^{51}Cr . Cytotoxicity was calculated using the same formula as for the NK cell assay.

Lymphocyte Blastogenesis

The lymphocyte preparations were resuspended in sufficient RPMI media to achieve a final concentration of

5×10^6 cells per 0.2 ml media. The suspensions were cultured in round bottom microtiter plates for 72 hours in the presence of 5 $\mu\text{g/ml}$ concanavalin A (ConA) or media alone. One microcurie of [^3H]-thymidine was added and the incubation continued for 16 additional hours. The cells were harvested and [^3H]-thymidine incorporation was measured by counting in a beta scintillation counter.

T Cells Subset Analysis

The lymphocyte preparations were assayed for the presence of pan T cells (OX-19), helper/inducer T cells (W3/25), and suppressor/cytotoxic T cells (OX-8). This determination was made using standard affinity purified fluorescent-labelled monoclonal antibody preparations. The labelled cells were counted on a fluorescence activated cell sorter.

Nucleic Acid Metabolism Assays

For the nucleic acid metabolism studies 1×10^7 lymphocytes were cultured in 1 ml of complete RPMI media in flat bottom polystyrene plates with and without ConA stimulation at 37°C in 5% CO_2 for 48 hours. The wells were then aspirated and the aspirate frozen at -70°C and thawed at room temperature three times. The resulting cell lysates were analyzed for adenosine deaminase activity and nucleotide triphosphate (NTP) levels. Adenosine deaminase activity was assayed using a colorimetric method described by Giusti [9]. Briefly, 0.05 ml aliquots of the thawed lymphocyte lysate were added to 1 ml of buffered adenosine solution (21 mM adenosine, 50 mM phosphate buffer, pH 6.5). After incubation for 60 minutes at 37°C, 3.0 ml of phenol-nitroprusside solution (106 mM phenol, 0.17 mM sodium nitroprusside) and 3.0 ml of alkaline hypochlorite (11 mM NaOCl, 125 mM NaOH) were added. After incubation for 30 minutes at 37°C, absorbance at 628 nm (E) was measured. Volume activity was determined as follows: (E sample - E sample blank) / (E standard - E reagent blank).

NTP was measured using a coupled enzymatic reaction [10]: $\text{NTP} + 3\text{-phosphoglycerate} \rightarrow \text{nucleotide diphosphate (NDP)} + 1,3\text{-diphosphoglycerate}$ [1] $1,3\text{-diphosphoglycerate} + \text{NADH} \rightarrow \text{glyceraldehyde 3-phosphate} + \text{nicotinamide-adenine dinucleotide (NAD)} + \text{phosphate}$ [2]. Reaction [1] was catalyzed by phosphoglycerate kinase and reaction [2] was catalyzed by glyceraldehyde phosphate dehydrogenase. The change in absorbance at 340 nm that occurs when NADH is oxidized to NAD was measured on a Gilford 240 spectrophotometer.

Glucose Metabolism

The rate of glucose metabolism was determined as follows. Lymphocytes were cultured at 1×10^7 lympho-

TABLE I. Survival Data of Rats Treated With Lactated Ringers or dPGE and Challenged With 1×10^6 Tumor Cells Subcutaneously, Intraperitoneally, or Intravenously

	Lactated Ringers	dPGE	P
Mean survival time with intraperitoneal tumor (days)	44.35 \pm 1.73	38.70 \pm 1.37	0.0063
Survival rate with intraperitoneal tumor	60%	15%	0.0033
Mean survival time with intravenous tumor (days)	39.84 \pm 1.85	34.40 \pm 0.85	0.0039
Mean survival time with intravenous tumor and 5 day delay in drug treatment (days)	39.06 \pm 1.13	37.33 \pm 1.67	0.8688
Mean survival time with subcutaneous tumor (days)	34.13 \pm 0.75	37.27 \pm 1.93	0.1408

cytes/ml in RPMI media with and without ConA stimulation as described in the preceding section. After 6, 18, and 48 hours of incubation, aspirates were obtained of the culture media and analyzed for glucose concentration. Glucose was determined on a Beckman Synchron CX3 system by means of the oxygen-rate method employing an oxygen electrode.

Statistical Analysis

All data are expressed as mean \pm standard error of the mean. Comparisons between groups were made using Fisher's exact test, a Wilcoxon test, and an unpaired T test. Significance was assumed at $P < 0.05$.

RESULTS

Survival data for the tumor models are shown in Table I. dPGE was not found to alter mean survival time when the tumor cell suspension was injected subcutaneously. dPGE administration significantly decreased both mean survival time ($P = 0.0063$) and absolute survival rate ($P = 0.0033$) in the peritoneal carcinomatosis model. With the pulmonary metastases model, when the dPGE administration was begun at the time of intravenous injection of the tumor cell suspension, it significantly decreased mean survival time ($P = 0.0039$). Administration of dPGE did not alter leukocyte infiltration of these pulmonary metastases. There were 17.0 ± 31.2 cells/hpf in the control group and 286.4 ± 25.5 cells/hpf in the treated group ($P = 0.167$). If the initiation of dPGE administration was delayed until 5 days following intravenous tumor challenge, there was no effect on mean survival time ($P = 0.8688$).

NK cell cytotoxicity was not altered by dPGE administration. The percent YAC cell lysis in the control group was $12.76 \pm 1.06\%$ and in the dPGE treated group $13.34 \pm 1.44\%$ ($P = 0.7710$) for 100:1 effector/target cell ratio. Macrophage cytotoxicity was also not significantly effected by dPGE administration. The percent YAC cell lysis in the control group was $31.56 \pm 4.03\%$

and in the dPGE treated group $29.83 \pm 3.70\%$ ($P = 0.7558$).

The data on the effect of dPGE administration on nucleic acid metabolism are shown in Table II. dPGE administration failed to alter spontaneous lymphocyte blastogenesis ($P = 0.7066$) or ConA stimulated blastogenesis ($P = 0.4175$). dPGE also failed to alter intracellular NTP concentrations in either unstimulated or ConA stimulated lymphocytes ($P = 0.0705$ and 0.9729 , respectively). Finally, dPGE did significantly reduce adenosine deaminase activity of both unstimulated and ConA stimulated lymphocytes ($P < 0.0001$ and $P < 0.0001$).

The effects of dPGE administration on the rate of glucose metabolism by lymphocytes are listed in Table III. It can be seen that dPGE administration did not significantly alter the rate of glucose consumption in nonstimulated lymphocytes. In contrast, dPGE administration significantly decreased the rate of glucose metabolism in ConA stimulated lymphocytes after both 18 and 48 hours of culture ($P < 0.0001$ and $P < 0.00001$, respectively).

The data on the effect of dPGE administration on T lymphocyte subset numbers are shown in Table IV. As can be seen, dPGE administration failed to alter the percentage of either pan T cells, helper/inducer T cells, or suppressor/cytotoxic T cells.

DISCUSSION

Patients with malignant neoplasms are known to have a number of physiologic and biochemical alterations. Among these is an increased level of activity of the cyclooxygenase enzyme system [1]. This leads to elevated levels of arachidonic acid metabolites, including PGE. Many of the physiologic alterations have been attributed to the elevated levels of PGE.

Certain of these alterations appear to be beneficial to the tumor host. Jaffe and Santoro have demonstrated that PGE decreases the rate of replication of multiple tumor cell lines [11]. This effect was noted whether the tumor

TABLE II. Nucleic Acid Metabolism Parameters of Lymphocytes Obtained From Rats Treated With Either Lactated Ringers or dPGE

	Lactated Ringers	dPGE	P
Unstimulated blastogenesis (cpm)	1,358 ± 112	1,277 ± 178	0.7066
ConA mitogen blastogenesis (cpm)	155,708 ± 21,209	134,186 ± 14,501	0.4175
Adenosine deaminase activity without ConA stimulation (units/10 ⁷ cells)	7.1 ± 0.5	2.5 ± 0.5	0.0001
Adenosine deaminase activity with ConA stimulation (units/10 ⁷ cells)	23.1 ± 2.4	3.6 ± 0.4	0.0001
NTP concentration without ConA stimulation (μg/10 ⁷ cells)	848.1 ± 158.8	453.1 ± 127.7	0.0705
NTP concentration with ConA stimulation (μg/10 ⁷ cells)	843.6 ± 84.8	849.5 ± 149.8	0.9729

TABLE III. Glucose Concentration (mg/dl) in RPMI Media Containing 1 × 10⁷ Lymphocytes/ml From Rats Treated With Either Lactated Ringers or dPGE

Incubation Time	Lactated Ringers	dPGE	P
Without ConA stimulation			
6 hours	191.9 ± 1.2	194.4 ± 0.8	0.1002
18 hours	200.2 ± 0.9	199.6 ± 1.3	0.7082
48 hours	197.3 ± 0.9	200.1 ± 1.1	0.0559
With ConA stimulation			
6 hours	200.2 ± 0.6	198.4 ± 0.7	0.0788
18 hours	187.6 ± 1.3	196.8 ± 1.3	0.0001
48 hours	152.4 ± 5.0	191.4 ± 1.4	0.00001

Accession For

NTIS GRAB

DTIC TAB

Unannounced

Justification

By

Distribution/

Availability Codes

Dist Avail and/or
Special

A-1

20



cell line was of a neural, epithelial, or fibroblastic nature. Prasad noted that PGE is able to increase the degree of differentiation of neuroblastoma cells [4]. The ability to both impair replication and induce tumor cell differentiation appear to be at least partially independent of each other [12].

Elevated levels of PGE may have other effects which are detrimental to oncology patients. PGE has been implicated in the development of certain osseous metastases. Bennett et al. noted a correlation between the level of PGE production by breast tumors and their rate of subsequent bony metastases [13]. Additional support for implicating PGE with bony metastases comes from the work of Powles et al. who demonstrated that administration of cyclooxygenase inhibitors to carcinosarcoma bearing rats decreased the incidence of bony metastases [14]. These findings were believed due to the fact that PGE causes demineralization of bone and thus creates a more favorable environment for successful tumor metastases to this location [15].

PGE may also affect tumor bearing patients by its ability to alter their immune system. PGE has been reported to impair immune function in multiple in vitro models, where PGE is added to cultures containing leukocytes obtained from normal healthy volunteers [16]. These impairments include a decreased lymphocyte blastogenesis, decreased interleukin-2 (IL2) production, in-

creased suppressor T lymphocyte activity, impaired NK cell cytotoxicity, and impaired macrophage cytotoxicity against tumor cells [6,7,17-20]. Similar immunologic abnormalities have been noted when studying leukocytes obtained from patients with various types of malignant tumors [21].

Utilizing the Wistar-Furth colon cancer cell line, we were unable to demonstrate any effect of dPGE on survival with our primary tumor model. In contrast, dPGE administration decreased survival time with both the peritoneal carcinomatosis model and the pulmonary metastasis model. However this effect was not seen when the initiation of dPGE therapy was delayed for 5 days, following tumor challenge. We interpret these findings to indicate that the PGE effect was probably not directly on the tumor cells but rather was more likely due to an alteration of some other component of the host response to the tumor cell. We therefore performed the immunologic assays described herein to determine if the decreased survival was due to an impaired immune response, as had been suggested by the prior in vitro work.

We were unable to demonstrate any adverse effect on macrophage or NK cell cytotoxicity when the dPGE was administered parenterally. We were also unable to demonstrate any effect of dPGE administration on other functional assays of lymphocytes including blastogenesis and T cell subset numbers. The failure of dPGE to alter

TABLE IV. Percentage of Splenic Pan T Cells, Helper/Inducer T Cells, or Suppressor/Cytotoxic T Cells in dPGE Treated and Lactated Ringers Treated Rats

	Lactated Ringers	dPGE	P
Percentage Pan T cells	60.2 ± 4.3%	61.2 ± 3.3%	0.8641
Percentage helper/inducer T cells	38.9 ± 2.3%	39.1 ± 1.2%	0.9429
Percentage suppressor/cytotoxic T cells	19.4 ± 1.1%	19.0 ± 1.3%	0.8273

the results of these immunologic parameters appears to suggest the possibility that the enhancement in tumor metastasis is not a result of impaired immune function, and would tend to question the validity of extrapolation of previous *in vitro* PGE data to *in vivo* situations.

Another seemingly contradictory series of findings in our data was our demonstration that dPGE administration decreased the rate of glucose consumption and the level of adenosine deaminase activity of lymphocytes. Previous reports would indicate that depression of these parameters should correlate with immunologic suppression. Hovi et al. have shown that adenosine deaminase activity is vital for lymphocyte proliferation [22]. Lum et al. noted increasing levels of adenosine deaminase activity correlated with enhanced immune function in renal transplant patients who were in the process of rejecting their allografts [23]. It has also been shown that addition of adenosine deaminase inhibitors impaired lymphocyte mediated cytotoxicity against tumor cells [24]. Our finding of depressed adenosine deaminase activity and glucose consumption without evidence of impairments in cytotoxicity against tumor cells may be explained by additional findings of Wolberg et al. [24]. They documented that although administration of adenosine deaminase inhibitors impaired lymphocyte mediated cytotoxicity against tumor cells, this impairment was of a brief duration lasting for only a few hours. Thereafter lymphocyte cytotoxicity returned to normal levels. It may therefore be that the dPGE caused a depression of adenosine deaminase activity which may have impaired leukocyte cytotoxicity during the initial hours following injection of tumor cells in our metastatic models. This period of time is obviously the most critical for a successful immunologic response to the blood borne tumor emboli. The dPGE-induced impairment in cytotoxicity could then have corrected itself during the ensuing days and could have been no longer measureable when we assayed NK cell and macrophage cytotoxicity after 1 week of drug treatment. Confirmation of this suggested hypothesis will require sequential early assays of leukocyte cytotoxicity during a prolonged course of parenteral dPGE administration.

An alternative explanation is that the dPGE administration enhanced tumor metastasis through a nonimmu-

nologic effect on the host. Specifically, this may have occurred through alterations in the endothelial cells of the pulmonary vasculature which enhanced the ability of the tumor emboli to bind to the endothelial cells, migrate between the cells, and establish successful colonies in the interstitium of the lung. A more thorough review of all such potential alterations has been previously published [25].

Wherever the effect of the dPGE was located, the difference in survival between those animals receiving dPGE at the time of intravenous tumor injection vs. those in which the dPGE was delayed for 5 days following tumor injection, indicates the critical nature of the time sequence of the host exposure to elevated levels of PGE in relation to tumor challenge. This also emphasizes the complex questions which need to be answered prior to initiating clinical trials of PGE analogs or inhibitors in surgical oncology patients.

ACKNOWLEDGMENTS

The authors would like to acknowledge Monica Plasek for secretarial assistance, and Jan Duke for library assistance in the preparation of this manuscript. This research was supported in part by NIH grant AG03417.

REFERENCES

1. Balch CM, Dougherty PA, Tilden AB: Excessive prostaglandin E₂ production by suppressor monocytes in head and neck cancer patients. *Ann Surg* 195:645-650, 1982.
2. Jaffe BM, LaRosa C, Kimura K: Prostaglandins and surgical diseases: part I. *Curr Probl Surg* 25:673-706, 1988.
3. Thomas DR, Philpott GW, Jaffe BM: The relationship between concentration of prostaglandin E and rates of cell replication. *Exp Cell Res* 84:40-46, 1974.
4. Prasad KN: Morphological differentiation induced by prostaglandin in mouse neuroblastoma cells culture. *Nature New Biol* 236:49-52, 1972.
5. Balch CM, Dougherty PA, Cloud GA, Tilden AB: Prostaglandin E₂-mediated suppression of cellular immunity in colon cancer patients. *Surgery* 95:71-77, 1984.
6. Brunda MJ, Herberman RB, Holden HT: Inhibition of murine natural killer cell activity by prostaglandins. *J Immunol* 124:2682-2687, 1980.
7. Schultz RM, Pavlidis NA, Stylos WA, Chirigos MA: Regulation of macrophage tumoricidal function: a role for prostaglandins of the E series. *Science* 202:320-321, 1978.
8. Waymack JP, Fernandes G, Yurt RW, Venkatraman JT, Burleson DG, Guzman RF, Mason AD Jr, Pruitt BA Jr: Effect of blood

- transfusions on immune function. Part VI. Effect on immunologic response to tumor. *Surgery* 108:172-178, 1990.
9. Giusti G: Adenosine deaminase determination. In Bergmeyer HU (ed): "Methods of Enzymatic Analysis." San Diego: Academic Press, Inc., 1974, Vol. 3, pp 1092-1099.
 10. Adams H: Adenosine 5'triphosphate determination with phosphoglycerate kinase. In Bergmeyer HU (ed): "Methods of Enzymatic Analysis." San Diego: Academic Press, Inc., 1965, Vol. 3, pp 539-543.
 11. Jaffe BM, Santoro ME: Prostaglandins and cancer. In Ramswell PW (ed): "T Prostaglandins." New York: Plenum Publishing Corp., 1977, Vol. 3, pp 329-351.
 12. Olsson IL, Breitman TR, Gallo RC: Priming of human myeloid leukemic cell lines HL-60 and U-937 with retinoic acid for differentiation effects of cyclic adenosine 3':5'-monophosphate-inducing agents and T-lymphocyte-derived differentiation factor. *Cancer Res* 42:3928-3933, 1982.
 13. Bennett A, Charlier EM, McDonald AM, Simpson JS, Stamford IF: Bone destruction by breast tumours. *Prostaglandins* 11:461-463, 1976.
 14. Powles TJ, Clark SA, Easty DM, Easty GC, Neville AM: The inhibition by aspirin and indomethacin of osteolytic tumor deposits and hypercalcaemia in rats with Walker tumour, and its possible application to human breast cancer. *Br J Cancer* 28:316-321, 1973.
 15. Klein DC, Raisz LG: Prostaglandins: stimulation of bone resorption in tissue culture. *Endocrinology* 86:1436-1440, 1970.
 16. Goldyne ME, Stobo JD: Immunoregulatory role of prostaglandins and related lipids. *Crit Rev Immunol* 2:189, 1981.
 17. Chouaib S, Chatenoud L, Klatzmann D, Fradelizi D: The mechanisms of inhibition of human IL 2 production. II. PGE₂ induction of suppressor T lymphocytes. *J Immunol* 132:1851-1857, 1984.
 18. Fulton AM, Levy JG: The possible role of prostaglandins in mediating immune suppression by nonspecific T suppressor cells. *Cell Immunol* 52:29-37, 1980.
 19. Goodwin JS, Messner RP, Peake GT: Prostaglandin suppression of mitogen-stimulated lymphocytes in vitro. Changes with mitogen dose and preincubation. *J Clin Invest* 62:753-760, 1978.
 20. Fischer A, Durandy A, Griscelli C: Role of prostaglandin E₂ in the induction of nonspecific T lymphocyte suppressor activity. *J Immunol* 126:1452-1455, 1981.
 21. Tilden AB, Balch CM: Indomethacin enhancement of immunocompetence in melanoma patients. *Surgery* 90:77-84, 1981.
 22. Hovi T, Smyth JF, Allison AC, Williams SC: Role of adenosine deaminase in lymphocyte proliferation. *Clin Exp Immunol* 23:395-403, 1976.
 23. Lum CT, Sutherland DE, Yasmin WG, Najarian JS: Peripheral blood mononuclear cell adenosine deaminase activity in renal allograft recipients. *J Surg Res* 24:388-395, 1978.
 24. Wolberg G, Zimmerman TP, Hiemstra K, Winston M, Chu LC: Adenosine inhibition of lymphocyte-mediated cytotoxicity: possible role of cyclic adenosine monophosphate. *Science* 187:957-959, 1975.
 25. Fidler IJ, Balch CM: The biology of cancer metastasis and implications for therapy. *Curr Probl Surg* 24:129-209, 1987.